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Research Article

Four new araneogenous species and a new genus in Hypocreales (Clavicipitaceae, Cordycipitaceae) from the karst region of China

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Abstract

The karst region in southwestern China is one of the biodiversity hotspots in the world with rich fungal diversity but under-studied. Four fungal species belonging to *Chlorocillium* (Clavicipitaceae) and *Gamszarella* (Cordycipitaceae) were isolated from dead spiders. Morphological comparisons, phylogenetic analyses and a PHI analysis based on multigene datasets support the establishment of these new species viz., *Chlorocillium guizhouense* **sp. nov.**, *C. vallense* **sp. nov.**, *Gamszarella sinensis* **sp. nov.**, and *G. vallensis* **sp. nov.** are introduced. A new genus, *Neogamszarella*, is proposed to accommodate *Gamszarella antillana*, which is phylogenetically distinct from *Gamszarella s. str*. Our results revealed that further attention needs to be paid to the diversity of araneogenous fungi in the karst regions of southwestern China.

Key words: *Chlorocillium*, lecanicillium-like, morphology, phylogenetic analysis, spider

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Introduction

Araneogenous or araneopathogenic fungi are spider-pathogenic fungi and are one of the ecologically important groups of fungi (Evans and Samson 1987). Formerly, araneogenous fungi were restricted to three families in Hypocreales: Cordycipitaceae (Kobayasi and Shimizu 1982; Chen et al. 2018; Mongkolsamrit et al. 2018) and Ophiocordycipitaceae (Kobayasi 1941; Samson and Evans 1975), and one species in Bionectriaceae (Chen et al. 2016a; Shrestha et al. 2019). However, Chen et al. (2022a) reported two new spider-associated genera in Clavicipitaceae. Wang et al. (2024a) reported *Akanthomyces* Lebert, *Beauveria* Vuill., *Cordyceps* Fr., *Engyodontium* de Hoog, *Gibellula* Cavara, *Hevansia* Luangsa-ard et al., *Lecanicillium* W. Gams & Zare, *Samsoniella* Mongkols. et al., *Torrubiella* Boud., *Jenniferia* Mongkols. et al., *Polystromomyces* Mongkols. et al., *Bhushaniella* Mongkolsamrit et al., *Hirsutella* Pat., *Hymenostilbe* Petch, *Ophiocordyceps* Petch, *Purpureocillium* Luangsa-ard et al., *Clonostachys* Corda and *Chlorocillium* Zare & W. Gams, from spiders. Crous et al. (2023) introduced a new araneogenous genus, *Gamszarella* Crous, while Khonsanit et al. (2024) introduced two new

spider-host genera, *Arachnidicola* Khons. et al., and *Corniculantispora* Khons., et al. and restricted the hosts of *Akanthomyces* to moths. Thus, the araneogenous fungi were distributed in 21 genera of Hypocreales (List of the genera see Suppl. material 1) (Crous et al. 2023; Khonsanit et al. 2024; Wang et al. 2024a).

The karst region in southwestern China is one of the 36 biodiversity hotspots in the world (Delgado-Baquerizo et al. 2020). A large range of continuously distributed primary forests exist in the karst region, with an exceptionally diverse ecosystem. As a result of the complex ecological environment and unique geographic conditions in this region, unique species can be described (Özkan et al. 2010; Su et al. 2017). Seventeen new araneogenous species and new records were reported by Chen et al. (2012, 2016a, b, 2017a, b, 2018, 2019a, 2022a, b, c, 2023, 2024), Han et al. (2013) and Zou et al. (2016, 2021) from karst region of southwest China.

During a survey of araneogenous fungi and their allies in southwestern China, infected spider specimens were collected, and fungal strains were isolated and purified. Isolated strains were identified based on the multigene phylogeny and morphological characteristics, and introduced four new species in Clavicipitaceae and Cordycipitaceae i.e. *Chlorocillium guizhouense* sp. nov., *C. vallense* sp. nov., *Gamszarella sinensis* sp. nov., and *G. vallensis* sp. nov. Moreover, *Gamszarella antillana* is not congeneric with *Gamszarella* s. str. in the phylogenetic analyses, thus, *Neogamszarella* is proposed to accommodate it.

Materials and methods

Specimen collection and identification

The specimens were collected from Dali Dong Village (26°01'58.70"N, 108°24'48.06"E), Rongjiang County, Qiandongnan Miao and Dong Autonomous Prefecture, Mayao River Valley (26°21'24.71"N, 107°22'48.22"E), Duyun City, Qiannan Buyi and Miao Autonomous Prefecture and Bala Valley (26°45'7.0344"N, 106°58'57.09"E), Wudang District, Guiyang, Guizhou Province, on 1st October 2018, 4th September 2021 and 5th April 2024, respectively. The samples were placed in an ice box and brought to the laboratory. Specimens were preserved in the refrigerator at 4 °C until further processing. The surface of each arthropod body was rinsed with sterile water, followed by sterilization with 75% ethanol for 3–5 s and rinsing again three times with sterilized water. After drying on sterilized filter paper, a piece of the synnema, mycelium or sclerotium was cut from the specimen and placed on plates of potato dextrose agar (PDA) or PDA modified by the addition of 1% w/v peptone containing 0.1 g/l streptomycin and 0.05 g/l tetracycline (Chen et al. 2019b). After fungal colonies emerged from the plated samples, a piece of mycelium from the colony edge was transferred onto new agar plates and cultured at 25 °C for 14 days under 12 h light/12 h dark conditions (Zou et al. 2010). The holotypes and ex-types cultures were deposited at the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China. MycoBank numbers were obtained as outlined in MycoBank (http://www.MycoBank.org) (Crous et al. 2004).

Colony characteristics were determined on PDA cultures incubated at 25 °C for 14 days and growth rate, presence of octahedral crystals and colony colors (surface and reverse) were observed. To investigate microscopic characteris-

tics, a little of the mycelia was picked up from the colony and mounted in lactophenol cotton blue or 20% lactic acid solution and the asexual morphological characteristics (e.g., conidiophores, phialides or conidiogenous cells, and conidia) were observed and measured using a Leica DM4 B microscope.

DNA extraction, polymerase chain reaction amplification and nucleotide sequencing

DNA extraction was carried out using a fungal genomic DNA extraction kit (DP2033, BioTeke Corporation) according to Liang et al. (2011). The extracted DNA was stored at −20 °C. Polymerase chain reaction (PCR) was used to amplify genetic markers using the following primer pairs: ITS4/ITS5 for the internal transcribed spacer (ITS) region (White et al. 1990), LR0R/LR5 for 28s large subunit ribosomal (LSU) (Vilgalys and Hester 1990), fRPB2-5F/fRPB2-7cR for RNA polymerase II second largest subunit (*RPB2*) (Liu et al. 1999) and 983F/2218R for translation elongation factor 1 alpha (*tef*-1α) (Castlebury et al. 2004). The thermal cycle of PCR amplification for these phylogenetic markers was set up following the procedure described by Chen et al. (2021). PCR products were purified and sequenced at Sangon Biotech (Shanghai) Co. All newly generated sequences were deposited in GenBank and accession numbers were obtained (Table 1).

Sequence alignments and phylogenetic analyses

DNASTAR™ Lasergene (v 6.0) was used to edit DNA sequences in this study. Analyses 1: ITS, LSU and *tef*-1α sequences for the strains in Clavicipitaceae were downloaded from GenBank based on Xiao et al. (2023), Chen et al. (2024) and other sequences were selected based on BLASTn searches. Analyses 2: ITS, LSU, *RPB2* and *tef*-1α sequences for the strains in Cordycipitaceae were downloaded from GenBank based on Khonsanit et al. (2024) and other sequences were selected based on BLASTn searches. All the sequences were aligned and edited by MAFFT v.7.037b (Katoh and Standley 2013) and MEGA6 (Tamura et al. 2013). Combined sequences for analysis 1 (dataset 1: ITS, LSU, *tef*-1α) and analysis 2 (dataset 2: ITS, LSU, *RPB2*, *tef*-1α) were obtained using SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The model was selected for Bayesian analysis by ModelFinder (Kalyaanamoorthy et al. 2017) in PhyloSuite (v1.2.2) software (Zhang et al. 2020).

The datasets 1 and 2 for analysis 1 and 2 were analyzed using Bayesian inference (BI) and maximum likelihood (ML) methods, respectively. For BI, a Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities for the combined sequence datasets using MrBayes v.3.2 (Ronquist et al. 2012). The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analysis, were discarded, while the remaining 16,001 trees were used to calculate posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined if it was greater than 200 using the program Tracer v.1.5 (Drummond and Rambaut 2007) to determine burn-in and confirm that both runs had converged. ML analyses were constructed with IQ-TREE (v 2.0) (Trifinopoulos et al. 2016), using an automatic selection of the model according to BIC.

Table 1. List of strains and GenBank accession numbers of sequences used in this study.

Species	Strain	GenBank Accession No.			
		ITS	LSU	*RPB2*	*tef*-1α
Aciculosporium oplismeni	MAFF 246966	LC571760	LC571760	-	LC572040
A. take	MAFF 241224	LC571753	LC571753	-	LC572034
A. take	TNS-F-60465	LC571755	LC571756	-	LC572035
Akanthomyces aculeatus	HUA 186145 ^{T}	-	MF416520	-	MF416465
A. aculeatus	TS 772	-	KC519370	-	KC519366
Aschersonia confluens	BCC 7961	JN049841	DQ384947	-	DQ384976
A. placenta	BCC 7869	JN049842	EF469074	-	EF469056
Ascopolyporus albus	BCC 48975 ^{T}	OL331502	OL322048	OL322065	OL322035
A. albus	BCC 48976	OL331503	OL322049	OL322066	OL322036
Arachnidicola sulphurea	TBRC 7248 ^{T}	NR_164419	MF140722	MF140812	MF140843
A. sulphurea	TBRC 7249	MF140757	MF140721	MF140734	MF140842
Atkinsonella hypoxylon	B4728	-	-	-	KP689546
Balansia epichloe	A.E.G. 96-15a	-	-	-	EF468743
B. henningsiana	A.E.G. 96-27a	JN049815	AY545727	-	AY489610
B. pilulaeformis	A.E.G. 94-2	-	AF543788	-	DQ522319
Beauveria bassiana	ARSEF 1564^{T}	HQ880761	-	HQ880905	HQ880974
B. bassiana	ARSEF 1478	AY531981	-	HQ880908	AY531890
Blackwellomyces cardinalis	OSC 93610	JN049843	AY184963	EF469106	EF469059
B. cardinalis	OSC93609^{T}	NR_159788	AY184962	DQ522422	DQ522325
Bhushaniella rubra	BCC 47541^{T}	OQ892128	OQ892133	OQ914433	OQ914428
B. rubra	BCC 47542	OQ892129	OQ892134	OQ914434	OQ914429
Chlorocillium araneogenum	DY101801^{T}	MW730536	MW730623	-	MW753039
C. araneogenum	DY101802	MW730545	MW730625	-	MW753040
C. griseum	CBS 387.73^{T}	KU382150	KU382218	-	-
C. griseum	RCEF6632	MW031768	MW084342	-	MW091327
C. gueriniae	BRIP 72680a^{T}	OR750699	OR731505	-	OR737799
C. gueriniae	BRIP 72666a	OR750701	OR731507	-	OR737801
C. gueriniae	BRIP 72668a	OR750702	OR731508	-	OR737802
C. lepidopterorum	SD05361^{T}	MW730543	MW730624	-	MW753041
C. lepidopterorum	SD05362	MW730611	MW730629	-	MW753042
C. montefioreae	BRIP 70299a^{T}	PP420202	PP415875	-	PP438400
C. guizhouense	**DL10171^{T}**	**MN128448**	**-**	-	**MN101596**
C. guizhouense	**DL10172**	**PQ432742**	**-**	-	**PQ444210**
C. sinense	KY07181^{T}	PP768154	PP768156	-	PP766580
C. sinense	KY07182	PP768155	PP768157	-	PP766581
C. vallense	**DY09021^{T}**	**PQ432743**	**PQ432746**	-	**PQ444211**
C. vallense	**DY09022**	**PQ432744**	**PQ432747**	-	**PQ444212**
Claviceps fusiformis	ATCC 26019	JN049817	U17402	-	DQ522320
C. purpurea	GAM 12885	U57669	AF543789	-	AF543778
C. purpurea	S.A. cp11	-	EF469075	-	EF469058
Clonostachys rosea	GJS90-227	-	AY489716	-	AY489611
Collarina aurantiaca	FMR 11134	KJ807178	KJ807181	-	-
C. aurantiaca	FMR 11784	KJ807177	KJ807180	-	-
Conoideocrella luteorostrata	NHJ 11343	JN049859	EF468850	-	EF468801
C. luteorostrata	NHJ 12516	JN049860	EF468849	-	EF468800
C. tenuis	NHJ 6293	JN049862	EU369044	-	EU369029
Corallocytostroma ornithocopreoides	WAC 8705	-	-	-	LT216546
Cordyceps militaris	OSC 93623 ^{T}	JN049825	AY184966	-	DQ522332
C. militaris	YFCC 6587	-	MN576818	MN576932	MN576988
C. inthanonensis	BCC 55812 ^{T}	MT000706	MT003041	MT017832	-

Species	Strain	GenBank Accession No.			
		ITS	LSU	*RPB2*	*tef*-1α
C. inthanonensis	BCC 56302	MT000705	MT003040	MT017831	MT017853
Corniculantispora psalliotae	CBS 532.81 ^{T}	MH861374	AF339560	EF469112	EF469067
C. psalliotae	CBS 101270	-	EF469081	EF469113	EF469066
Corpulentispora magnispora	CGMCC 3.19304 ^{T}	MK329102	MK329007	MK335985	MK336037
C. magnispora	LC12469	MK329103	MK329008	MK335986	MK336038
*Dussiella tuberiformis**		-	-	-	JQ257027
Engyodontium aranearum	CBS 309.85	JN036556	AF339526	DQ522439	DQ522341
Ephelis japonica	CBS 236.64	MH858427	-	-	-
E. japonica	Eph.oryzae	AB038564	-	-	-
E. tripsaci	CBS 857.72^{T}	NR_153997	NG_059240	-	-
E. elymi	C. Schardl 760	-	AY986924	-	AY986951
Epichloe typhina	ATCC 56429	JN049832	U17396	-	AF543777
Flavocillium acerosum	CBS 418.81^{T}	NR_111268	KM283786	KM283852	KM283810
F. bifurcatum	YFCC 6101^{T}	MN576833	MN576781	MN576897	MN576951
Gamszarea humicola	CGMCC 3.19303^{T}	NR_172830	NG_075268	MK335979	MK336027
G. wallacei	CBS 101237^{T}	NR_111267	NG_042398	EF469119	EF469073
Gamszarella buffelskloofina	CBS 150062^{T}	OR680769	OR717025	OR683726	
G. sinensis	**WD04081^{T}**	**PQ527895**	**PQ527899**	**PQ553218**	**PQ553220**
G. sinensis	**WD04082**	**PQ527896**	**PQ527900**	**PQ553219**	**PQ553221**
G. vallensis	**WD04101^{T}**	**PQ527897**	**PQ527901**	-	-
G. vallensis	**WD04102**	**PQ527898**	**PQ527902**	-	-
Gibellula nigeli	NHJ 10808^{T}	-	EU369035	EU369076	EU369018
G. nigeli	BCC47555	MH532885	-	-	MH521897
Helicocollum surathaniensis	BCC 34463	-	KT222328	-	KT222336
H. surathaniensis	BCC 34464^{T}	-	KT222329	-	KT222337
Heteroepichloe bambusae	Ba-01	AB065426	-	-	-
H. bambusae	Bo-01	AB065428	-	-	-
H. sasae	*E.sasae*-H	AB065432	-	-	-
H. sasae	*E.sasae*-N	AB065431	-	-	-
Hevansia novoguineensis	CBS 610.80^{T}	NR_169678	NG_070837	MH521844	MH521885
H. novoguineensis	BCC 47881	JX192685	MH394650	MH521845	MH521886
Hyperdermium bertonii	AF242354	-	AF242354	-	-
Jenniferia cinerea	BCC 06839	GQ249999	GQ249970	EU369070	EU369009
J. cinerea	BCC 2191	GQ250000	GQ249971	-	GQ250029
Kanoksria zaquensis	HMAS 246915^{T}	MT789699	MT789697	-	MT797812
K. zaquensis	HMAS 246917	MT789698	MT789696	-	MT797811
Keithomyces carneus	CBS 239.32	NR_131993	NG_057769	-	EF468789
Lecanicillium lecanii	CBS 101247	JN049836	AF339555	DQ522466	DQ522359
L. lecanii	CBS 102067^{T}	MH862778	KM283795	KM283860	KM283818
Leptobacillium chinense	CGMCC 3.14970^{T}	NR_155782	NG_069101	-	-
L. coffeanum	COAD 2057^{T}	MF066035	MF066033	-	-
L. coffeanum	COAD 2061	MF066034	MF066032	-	-
L. filiforme	URM 7918^{T}	NR_171744	NG_075252	-	-
L. leptobactrum	CBS 774.69^{T}	NR_154111	NG_069745	-	-
Liangia sinensis	YFCC 3103^{T}	MN576831	MN576782	MN576898	MN576952
L. sinensis	YFCC 3104	MN576832	MN576783	MN576899	MN576953
Marquandomyces marquandii	CBS 182.27	NR_131994	EF468845	-	EF468793
Metapochonia bulbillosa	CBS 145.70	MH859529	AF339542	-	EF468796
M. gonioides	CBS 891.72	AJ292409	AF339550	-	DQ522354
M. rubescens	CBS 464.88^{T}	-	AF339566	-	EF468797
M. sulchlasporia	CBS 251.83	NR_154139	MH873311	-	KJ398790

Species	Strain	GenBank Accession No.			
		ITS	LSU	*RPB2*	*tef*-1α
Metarhiziopsis microspora	CEHS133a	EF464589	EF464571	-	-
M. microspora	INEHS133a	EF464583	EF464572	-	-
Metarhizium anisopliae	ARSEF 7487	HQ331446	-	-	DQ463996
M. anisopliae	CBS 130.71^{T}	MT078884	MT078853	-	MT078845
M. flavoviride	CBS 125.65	MT078885	MT078854	-	MT078846
M. flavoviride	CBS 700.74	-	MT078855	-	MT078847
M. flavoviride	CBS 218.56^{T}	MH857590	MH869139	-	KJ398787
Microhilum oncoperae	ARSEF 4358	-	AF339532	EF468936	EF468785
Moelleriella phyllogena	CUP 067785	-	EU392610	-	EU392674
M. phyllogena	CUP 067793	-	EU392608	-	EU392672
M. umbospora	CUP 067817^{T}	-	EU392628	-	EU392688
Morakotia fusca	BCC 64125	-	KY794862	-	KY794857
M. fusca	BCC 79272^{T}	-	KY794861	-	KY794856
Mycophilomyces periconiae	CPC 27558	NR_154209	NG_059746	-	-
Myriogenospora atramentosa	A.E.G 96-32	-	AY489733	-	AY489628
Neoaraneomyces araneicola	DY101711^{T}	MW730520	MW730609	-	MW753033
N. araneicola	DY101712	MW730522	MW730610	-	MW753034
Neobaryopsis andensis	A.F.25967-2^{T}	NR_169713	NG_068701	-	-
N. andensis	A.F.25967-1	MT153956	MT153985	-	-
Neobarya parasitica	Marson s/n	KP899626	KP899626	-	-
Neogamszarella antillata	CBS 350.85^{T}	NR_111097	AF339536	DQ522450	DQ522350
Neohyperdermium piperis	CBS 116719^{T}	-	AY466442	EU369083	DQ118749
N. pulvinatum	P.C. 602	-	DQ118738	AF242353	DQ118746
Neotorrubiella chinghridicola	BCC 80733^{T}	NR_175629	MK632097	MK632149	-
N. chinghridicola	BCC 39684	MK632038	MK632096	-	MK632072
Niesslia exilis	CBS 560.74	-	AY489720	-	AY489614
Nigelia aurantiaca	BCC13019	-	GU979948	-	GU979957
N. martiale	EFCC 6863	-	JF415974	-	JF416016
Niveomyces coronatus	NY04434800^{T}	-	ON493606	ON513400	ON513397
N. coronatus	Niveo	-	ON493605	-	-
Orbiocrella petchii	NHJ 6209	JN049861	EU369039	-	EU369023
O. petchii	NHJ 6240	-	EU369038	-	EU369022
Papiliomyces liangshanensis	EFCC 1452	-	EF468815	-	EF468756
P. liangshanensis	EFCC 1523	-	EF468814	-	EF468755
P. shibinensis	GZUH SB13050311^{T}	NR154178	-	-	KR153589
Parahevansia koratensis	BCC01485	GQ250010	GQ249981	-	GQ250031
P. websteri	NHJ 2662	GQ250008	GQ249982	-	GQ250032
Parametarhizium changbaiense	CGMCC 19143^{T}	MN589741	MN589994	-	MN908589
P. hingganense	CGMCC 19144	MN055703	MN061635	-	MN065770
Paraneoaraneomyces sinensis	ZY 22.006	OQ709254	OQ709260	-	OQ719626
P. sinensis	ZY 22.007	OQ709255	OQ709261	-	OQ719627
Parengyodontium album	CBS 836.71	LC092882	MH872118	-	LC382178
P. album	CBS 368.72	MH860502	MH872217	-	LC382183
Parepichloe cinerea	Ne-01	AB065425	-	-	-
Periglandula ipomoeae	IasaF13	-	-	-	KP689568
Pleurodesmospora coccorum	CBS 460.73	MH860743	MH872455	-	-
P. lepidopterorum	DY 10501^{T}	MW826576	-	MW834316	MW834317
P. lepidopterorum	DY 10502	MW826577	-	MW834318	MW834319
Pochonia boninensis	JCM 18597	AB709858	AB709831	-	AB758463
P. chlamydosporia	CBS 101244	JN049821	DQ518758	-	DQ522327
Polystromomyces araneae	BCC 93301^{T}	MZ684101	MZ684016	MZ707845	MZ707825

Species	Strain	GenBank Accession No.			
		ITS	LSU	*RPB2*	*tef*-1α
Pseudogibellula formicarum	BCC 84257	MT508782	MT512653	-	MT533480
P. formicarum	CBS 433.73	MH860731	MH872442	-	MT533481
Pseudoniveomyces arachnovorum	BCC 95818^{T}	OR098526	-	-	OR133173
P. blattae	BCC 53567^{T}	ON103042	ON103167	ON125036	-
P. blattae	BCC 53568	ON103043	ON103168	ON125037	ON125025
Pseudolecanicillium caatingaense	URM8446^{T}	ON862933	ON862925	OP290514	OP290526
P. caatingaense	URM8442	ON862934	ON862926	OP290513	OP290525
Purpureomyces maesotensis	BCC 88441	MN781916	MN781877	-	MN781734
P. maesotensis	BCC 85349	MN781928	MN781872	-	MN781729
P. maesotensis	BCC 89300^{T}	MN781917	MN781876	-	MN781733
Regiocrella camerunensis	ARSEF 7682	-	DQ118735	-	DQ118743
Rotiferophthora angustispora	CBS 101437	AJ292412	AF339535	-	AF543776
Samsoniella alboaurantium	CBS 262.58^{T}	AY624179	AB080087	MF416448	MF416497
S. alboaurantium	CBS 240.32	AY624178	JF415979	JF415999	JF416019
S. inthanonensis	TBRC 7915^{T}	NR_164420	NG_069500	MF140815	MF140849
S. inthanonensis	TBRC 7916	MF140760	MF140724	MF140814	MF140848
Samuelsia chalalensis	CUP 067856^{T}	-	EU392637	-	EU392691
S. mundiveteris	BCC 40021	-	GU552152	-	GU552145
S. rufobrunnea	CUP 067858	-	AY986918	-	AY986944
Shimizuomyces paradoxus	EFCC 6279	JN049847	EF469084	-	EF469071
S. paradoxus	EFCC 6564	-	EF469083	-	EF469072
Simplicillium lanosoniveum	CBS 123.42^{T}	NR_171734	NG_068571	-	-
Sungia yongmunensis	EFCC 2131	JN049856	EF468833	-	EF468770
S. yongmunensis	EFCC 2135	-	EF468834	-	EF468769
Tyrannicordyceps fratricida	TNS-F 19011	JQ349068	JQ257023	-	JQ257028
Ustilaginoidea dichromonae	MRL IB9228	-	-	-	JQ257025
U. virens	ATCC 16180	-	-	-	JQ257026
U. virens	MAFF 240421	-	JQ257011	-	JQ257024
Yosiokobayasia kusanagiensis	TNS-F18494	-	JF415972	-	JF416014
Zarea fungicola	CBS 992.69^{T}	NR_119653	KM283792	KM283857	KM283816
Zouia cauligalbarum	GZUIFRZHJ01^{T}	MH730663	MH730667	MH801924	MH801920
Z. cauligalbarum	GZUIFRZHJ02	MH730664	MH730668	MH801925	MH801921
Pleurocordyceps aurantiaca	MFLUCC 17-2113	MG136916	MG136910	-	MG136875
P. marginaliradians	MFLU 17-1582^{T}	MG136920	MG136914	-	MG136878
Purpureocillium lilacinum	CBS 431.87	AY624188	EF468844	EF468940	EF468791

Note: New strains or species are in bold type. * J.F. White, Scale on Arundinaria tecta, North Carolina, 2000. "^{T}" denotes ex-type. Abbreviations: ARSEF, USDA-ARS Collection of Entomopathogenic Fungal cultures, Ithaca, NY; ATCC, American Type Culture Collection, USA; BCC, BIOTEC Culture Collection, KlongLuang, Thailand; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CGMCC, China General Microbiological Culture Collection Center, China; EFCC, Entomopathogenic Fungal Culture Collection, Chuncheon, Korea; GZUH, Guizhou University Herbarium, Guiyang, Guizhou, China; HMAS, Herbarium of Mycology, Chinese Academy of Sciences; MAFF, Ministry of Agriculture, Forestry and Fisheries of Japan, Tokyo, Japan; NHJ, Nigel Hywel-Jones personal collection; OSC, Oregon State University Herbarium, Corvallis, OR; TNS-F, the mycological herbarium of the National Museum of Nature and Science, Tsukuba, Ibaraki, Japan; WAC, Western Australian Plant Pathology Reference Culture Collection, Australia, Perth.

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) analysis

The Genealogical Concordance Phylogenetic Species Recognition model was applied to analysis the related species. The pairwise homoplasy index (PHI) (Bruen et al. 2006) is a model test based on the fact that multiple gene phylogenies will be concordant between species and discordant due to recombination and mutations within a species. The test was performed in SplitsTree4 (Huson and Bryant 2006) as described by Quaedvlieg et al. (2014) to determine

the recombination level within phylogenetically closely-related species using a three-locus or four-locus concatenated dataset. The new species and their closely-related species were analyzed using this model. The relationships between closely-related species were visualized by constructing a split graph, using both the LogDet transformation and splits decomposition options.

Results

Phylogenetic analyses

Analysis 1: Phylogenetic trees were generated in analysis 1 to determine the establishment of the new *Chlorocillium* species in Clavicipitaceae (Fig. 1). *Pleurocordyceps aurantiaca* (Y.P. Xiao et al.) Y.H. Wang et al. (MFLUCC 17-2113) and *P. marginaliradians* (Y.P. Xiao et al.) Y.H. Wang et al. (MFLU 17-1582) were used as the outgroup taxa in the analysis. The dataset included 74 taxa, and consisted of 2,280 (ITS, 660; LSU, 740, and *tef*-1α, 880) characters with gaps.

The selected model for ML analysis was TIM+F+I+G4. The final value of the highest scoring tree was −26,825.060, which was obtained from the ML analysis of the dataset. The parameters of the GTR model used to analyze the dataset were estimated based on the following frequencies: A = 0.224, C = 0.291, G = 0.279, T = 0.207; substitution rates AC = 1.00000, AG = 2.11049, AT = 1.16800, CG = 1.16800, CT = 5.30232 and GT = 1.00000, as well as the gamma distribution shape parameter α = 0.474. The selected model of the dataset for BI analysis was GTR+F+I+G4. The phylogenetic tree (Fig. 1) constructed using ML and BI analyses was largely congruent and strongly supported in most branches. Most genera clustered into independent clades. Strains DL10171, DL10172, DY09021 and DY09022 clustered into two independent clades. Strains DL10171 and DL10172 have a close relationship with *Chlorocillium griseum*, whereas strains DY09021 and DY09022 have a close relationship with *C. sinense* W.H. Chen et al. and *C. araneogenum* (W.H. Chen et al.) W.H. Chen, et al.

Analysis 2: The phylogenetic trees were generated in analysis 2 to determine the establishment of the new species in Cordycipitaceae (Fig. 2). *Purpureocillium lilacinum* (Thom) Luangsa-ard et al. (CBS 431.87) was used as the outgroup taxon in the analysis. The dataset included 49 taxa and consisted of 2,280 (ITS, 631; LSU, 782, *RPB2*, 833 and *tef*-1α, 893) characters with gaps.

The selected model for ML analysis was TIM3+F+I+G4. The final value of the highest scoring tree was −36,309.393, which was obtained from the ML analysis of the dataset. The parameters of the GTR model used to analyze the dataset were estimated based on the following frequencies: A = 0.237, C = 0.280, G = 0.272, T = 0.211; substitution rates AC = 1.16611, AG = 2.87992, AT = 1.00000, CG = 1.16611, CT = 6.74648 and GT = 1.00000, as well as the gamma distribution shape parameter α = 0.445. The selected model for BI analysis was GTR+F+I+G4 (ITS), GTR+F+I+G4 (LSU and *tef*-1α) and SYM+G4 (*RPB2*). The phylogenetic trees (Fig. 2) constructed using ML and BI analyses were largely congruent and strongly supported in most branches. Most genera clustered into independent clades. Strains WD04081, WD04082, WD04101 and WD04102 clustered into two independent clades with high statistical support (100% ML/1 PP) and were clustered with *Gamszarella antillana* (R.F. Castañeda & G.R.W. Arnold) Crous and *G. buffelskloofina* Crous in a clade with high statistical support in ML and BI analysis (100% ML/1 PP).

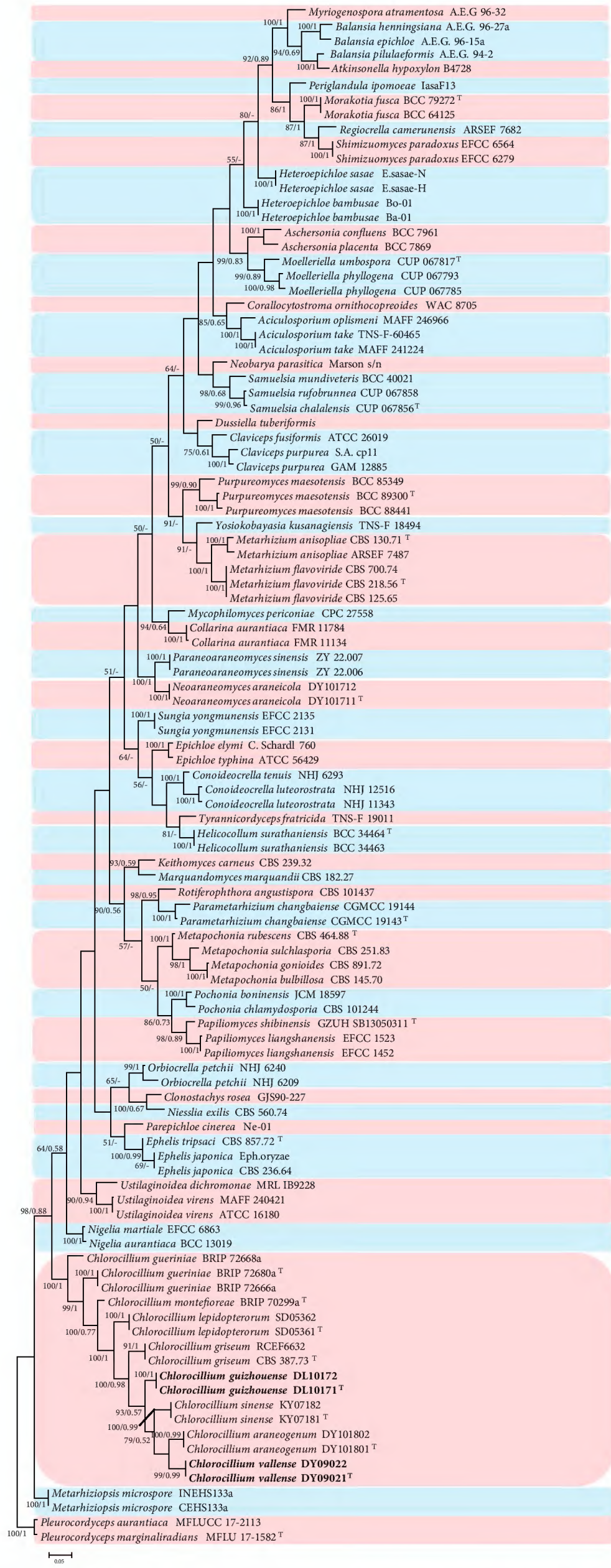

Figure 1. Phylogram retrieved from IQTREE of the new species and related species in Clavicipitaceae using the combined dataset of ITS, LSU, and *tef*-1α gene regions. The statistical values are provided at nodes as ML/PP (ML value above 50% and BI value above 0.50). The tree is rooted with *Pleurocordyceps aurantiaca* (MFLUCC 17-2113) and *P. marginaliradians* (MFLU 17-1582). Ex-types, new strains and new species are indicated by the superscript "T" and in bold, respectively.

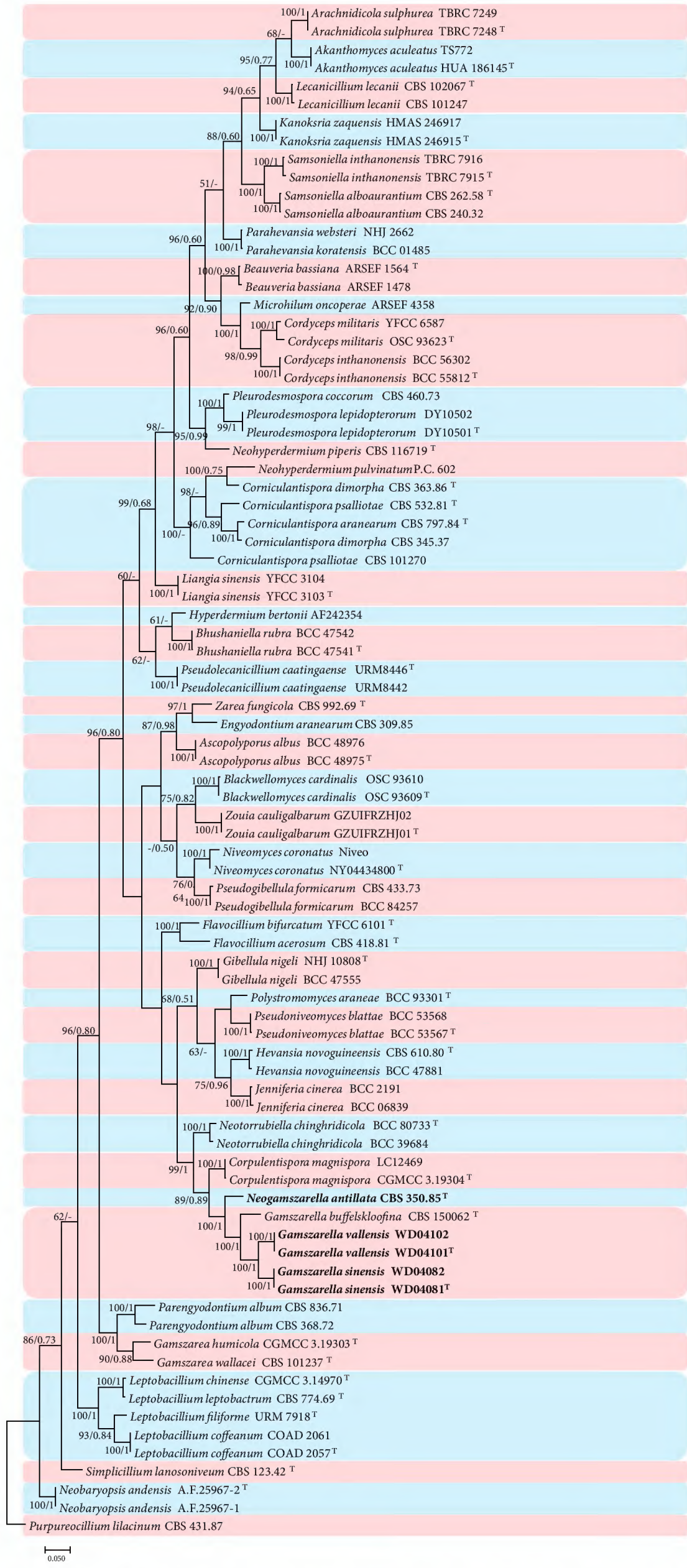
Arachnidicola sulphurea TBRC 7249
Arachnidicola sulphurea TBRC 7248 T
Akanthomyces aculeatus TS772
Akanthomyces aculeatus HUA 186145 T
Lecanicillium lecanii CBS 102067 T
Lecanicillium lecanii CBS 101247
Kanoksria zaquensis HMAS 246917
Kanoksria zaquensis HMAS 246915 T
Samsoniella inthanonensis TBRC 7916
Samsoniella inthanonensis TBRC 7915 T
Samsoniella alboaurantium CBS 262.58 T
Samsoniella alboaurantium CBS 240.32
Parahevansia websteri NHJ 2662
Parahevansia koratensis BCC 01485
Beauveria bassiana ARSEF 1564 T
Beauveria bassiana ARSEF 1478
Microhilum oncoperae ARSEF 4358
Cordyceps militaris YFCC 6587
Cordyceps militaris OSC 93623 T
Cordyceps inthanonensis BCC 56302
Cordyceps inthanonensis BCC 55812 T
Pleurodesmospora coccorum CBS 460.73
Pleurodesmospora lepidopterorum DY10502
Pleurodesmospora lepidopterorum DY10501 T
Neohyperdermium piperis CBS 116719 T
Neohyperdermium pulvinatum P.C. 602
Corniculantispora dimorpha CBS 363.86 T
Corniculantispora psalliotae CBS 532.81 T
Corniculantispora aranearum CBS 797.84 T
Corniculantispora dimorpha CBS 345.37
Corniculantispora psalliotae CBS 101270
Liangia sinensis YFCC 3104
Liangia sinensis YFCC 3103 T
Hyperdermium bertonii AF242354
Bhushaniella rubra BCC 47542
Bhushaniella rubra BCC 47541 T
Pseudolecanicillium caatingaense URM8446 T
Pseudolecanicillium caatingaense URM8442
Zarea fungicola CBS 992.69 T
Engyodontium aranearum CBS 309.85
Ascopolyporus albus BCC 48976
Ascopolyporus albus BCC 48975 T
Blackwellomyces cardinalis OSC 93610
Blackwellomyces cardinalis OSC 93609 T
Zouia cauligalbarum GZUIFRZHJ02
Zouia cauligalbarum GZUIFRZHJ01 T
Niveomyces coronatus Niveo
Niveomyces coronatus NY04434800 T
Pseudogibellula formicarum CBS 433.73
Pseudogibellula formicarum BCC 84257
Flavocillium bifurcatum YFCC 6101 T
Flavocillium acerosum CBS 418.81 T
Gibellula nigeli NHJ 10808 T
Gibellula nigeli BCC 47555
Polystromomyces araneae BCC 93301 T
Pseudoniveomyces blattae BCC 53568
Pseudoniveomyces blattae BCC 53567 T
Hevansia novoguineensis CBS 610.80 T
Hevansia novoguineensis BCC 47881
Jenniferia cinerea BCC 2191
Jenniferia cinerea BCC 06839
Neotorrubiella chinghridicola BCC 80733 T
Neotorrubiella chinghridicola BCC 39684
Corpulentispora magnispora LC12469
Corpulentispora magnispora CGMCC 3.19304 T
Neogamszarella antillata CBS 350.85 T
Gamszarella buffelskloofina CBS 150062 T
Gamszarella vallensis WD04102
Gamszarella vallensis WD04101 T
Gamszarella sinensis WD04082
Gamszarella sinensis WD04081 T
Parengyodontium album CBS 836.71
Parengyodontium album CBS 368.72
Gamszarea humicola CGMCC 3.19303 T
Gamszarea wallacei CBS 101237 T
Leptobacillium chinense CGMCC 3.14970 T
Leptobacillium leptobactrum CBS 774.69 T
Leptobacillium filiforme URM 7918 T
Leptobacillium coffeanum COAD 2061
Leptobacillium coffeanum COAD 2057 T
Simplicillium lanosoniveum CBS 123.42 T
Neobaryopsis andensis A.F.25967-2 T
Neobaryopsis andensis A.F.25967-1
Purpureocillium lilacinum CBS 431.87
0.050

Figure 2. Phylogram retrieved from IQTREE of the new species and other related species in Cordycipitaceae using the combined dataset of ITS, LSU, *RPB2* and *tef*-1α gene regions. The statistical values are provided at nodes as ML/PP (ML value above 50% and BI value above 0.50). The tree is rooted with *Purpureocillium lilacinum* (CBS 431.87). Ex-types, new strains and new species are indicated by the superscript "T" and in bold, respectively.

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) analysis

A three-locus concatenated dataset (ITS, LSU and *tef*-1α) was used to determine the recombination level within *Chlorocillium araneogenum* (DY101801), *C. griseum* (CBS 387.73), *C. gueriniae* (BRIP 72680a), *C. montefioreae* (BRIP70299a), *C. lepidopterorum* (SD05361), *C. guizhouense* (DL10171), and strains KY07181 and DY09021 (Fig. 3), whereas a four-locus concatenated dataset (ITS, LSU, *RPB2* and *tef*-1α) was used to determine the recombination level within *Gamszarella antillana* (CBS 350.85), *G. buffelskloofina* (CBS 150062), and strains WD04081 and WD04101 (Fig. 4). Chaiwan et al. (2022) noted that if

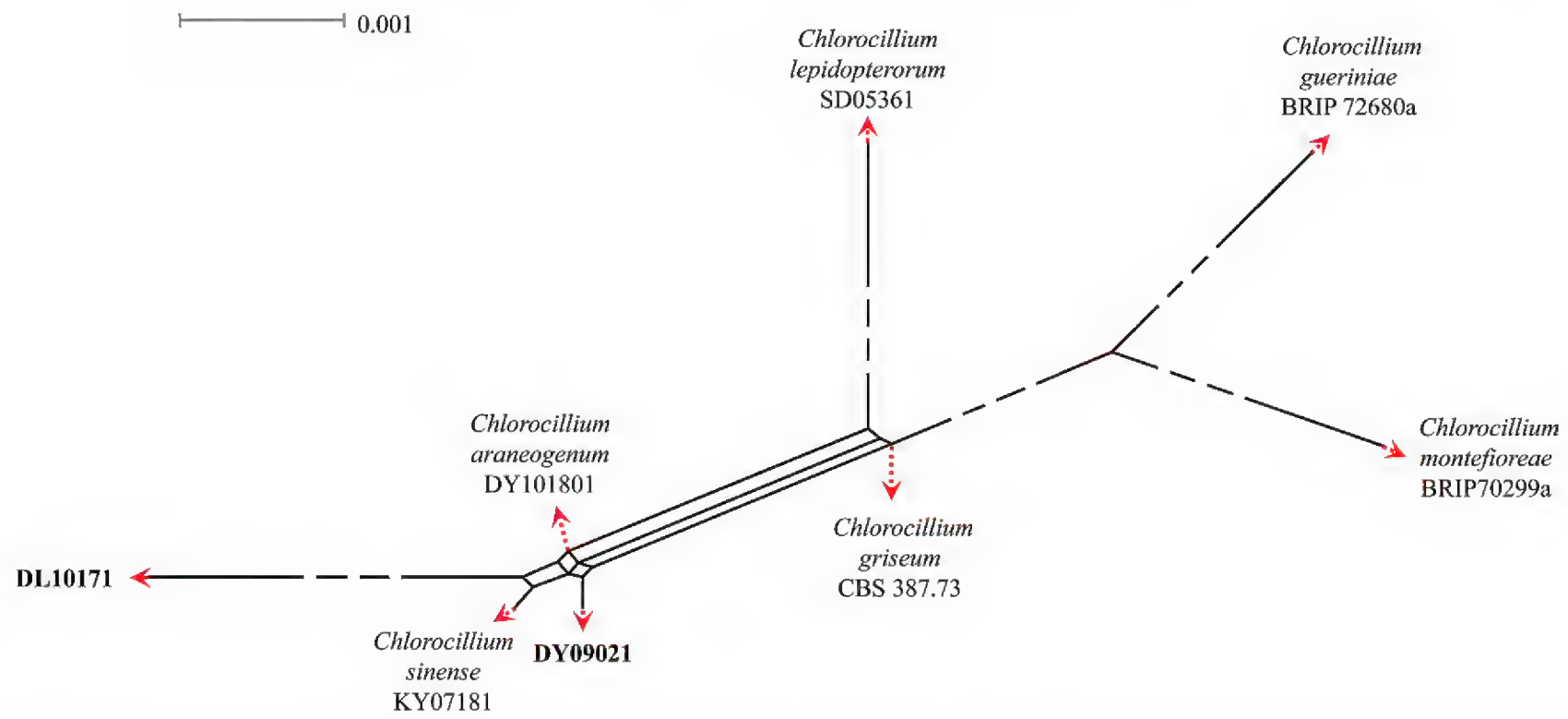

Figure 3. Results of the pairwise homoplasy index (PHI) test of the new *Chlorocillium* strains and its closely-related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset. The new strains are in bold type.

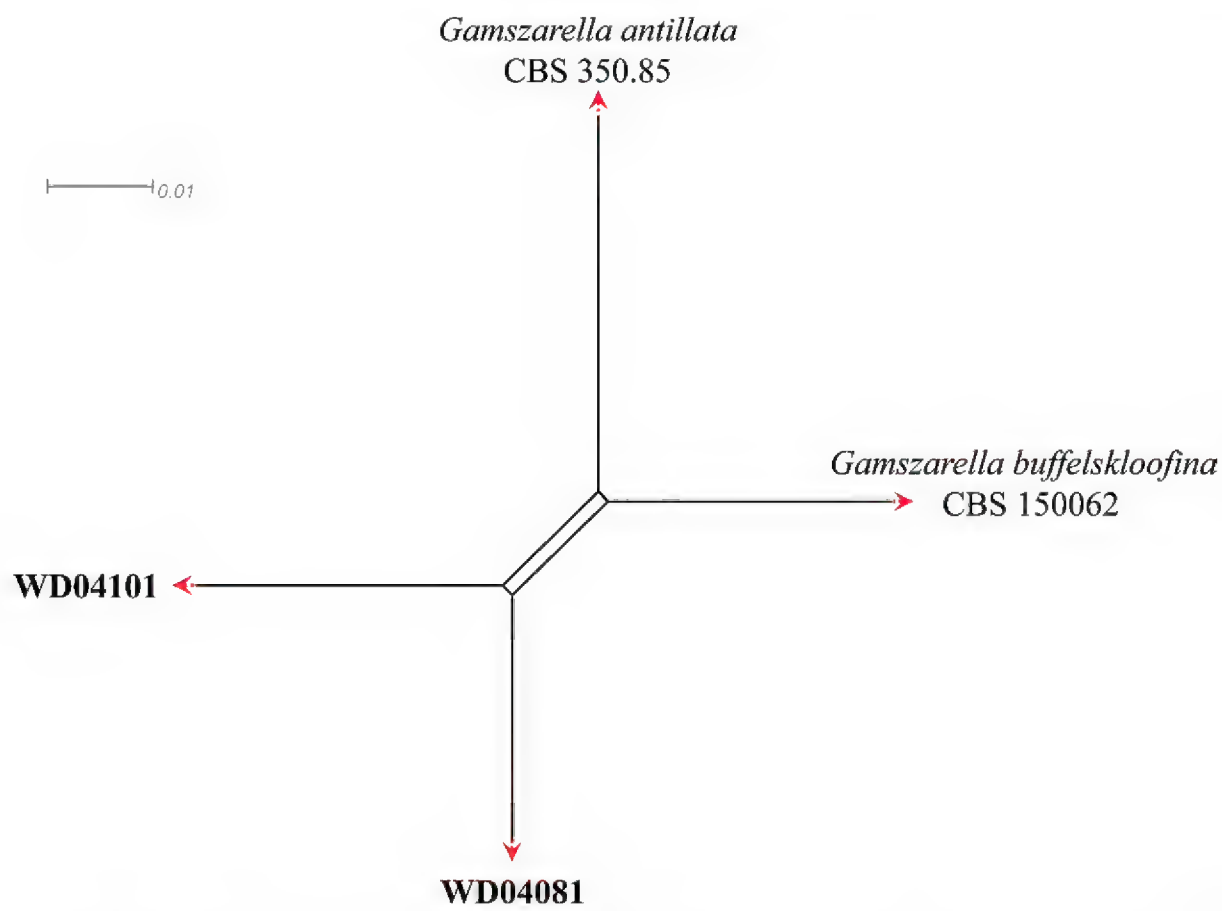

Figure 4. Results of the pairwise homoplasy index (PHI) test of the new *Gamszarella* strains and its closely-related species using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset. The new strains are in bold type.

the PHI is below the 0.05 threshold (Φ_w < 0.05), it indicates that there is significant recombination in the dataset. This means that related species in a group and recombination levels are not different. If the PHI is above the 0.05 threshold (Φ_w > 0.05), it indicates that it is not significant, which means that the related species in a group level are different. The result of the pairwise homoplasy index (PHI) test of *Chlorocillium araneogenum*, *C. griseum*, *C. gueriniae*, *C. montefioreae*, *C. lepidopterorum*, *C. guizhouense*, strains KY07181 and DY09021 was 1.0 and revealed that those species and strains KY07181 and DY09021 were different (Fig. 3). The result of the pairwise homoplasy index (PHI) test of *Gamszarella antillana*, *G. buffelskloofina*, and strains WD04081 and WD04101 was 1.0 and revealed that those species and strains WD04081 and WD04101 were different (Fig. 4).

Taxonomy

Chlorocillium **Zare & W. Gams, Mycol. Progr. 15: 1005, 2016**

Chlorocillium guizhouense **W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.**

MycoBank No: 856175

Fig. 5

Etymology. Referring to the location, Guizhou Province, where the type specimen was collected.

Type. China • Guizhou Province, Qiandongnan Miao and Dong Autonomous Prefecture, Rongjiang County, Dali Dong Village (26°01'58.70"N, 108°24'48.06"E). On a dead spider (Araneae), 1 October 2018, Wanhao Chen, GZAC DL1017 (holotype), ex-type, DL10171.

Description. Colonies on PDA reaching 21–24 mm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.6 µm diam. Conidial structures consisting of erect conidiophores usually arising from the aerial hyphae, solitary or lecanicillium-like with phialides in whorls of two to three. Phialides 11.6–25.3 × 1.0–1.2 µm, with a cylindrical basal portion, tapering into a distinct neck. Conidia in chains, hyaline, fusiform, 1-celled, 2.6–3.8 × 1.1–1.6 µm (average values 3.2 × 1.3 µm). Octahedral crystals not observed.

Host. Spider (Araneae).

Additional material examined. China • Guizhou, Qiandongnan Miao and Dong Autonomous Prefecture, Rongjiang County, Dali Dong Village (26°01'58.70"N, 108°24'48.06"E). On a dead spider (Araneae), 1 October 2018, Wanhao Chen, DL10172 (living culture).

Remarks. *Chlorocillium guizhouense* was identified as *Chlorocillium*, based on the BLASTn result in NCBI and the phylogenetic analysis of the combined dataset 1 (Fig. 1). It clustered into an independent clade with a close relationship with *C. araneogenum*, *C. sinense and C. vallense* with high bootstrap value (93% ML). Compared with the typical characteristics of the known species (Table 2), *C. guizhouense* was distinguished from *C. araneogenum* by its smaller conidia (2.6–3.8 × 1.1–1.6 µm vs. 3.4–5.8 × 1.4–1.8 µm). *Chlorocillium guizhouense* can be distinguished from *C. sinense* by its larger conidia (fusiform, 2.6–3.8 × 1.1–1.6 µm vs. fusiform to ellipsoidal, 1.9–2.9 × 0.8–1.2 µm).

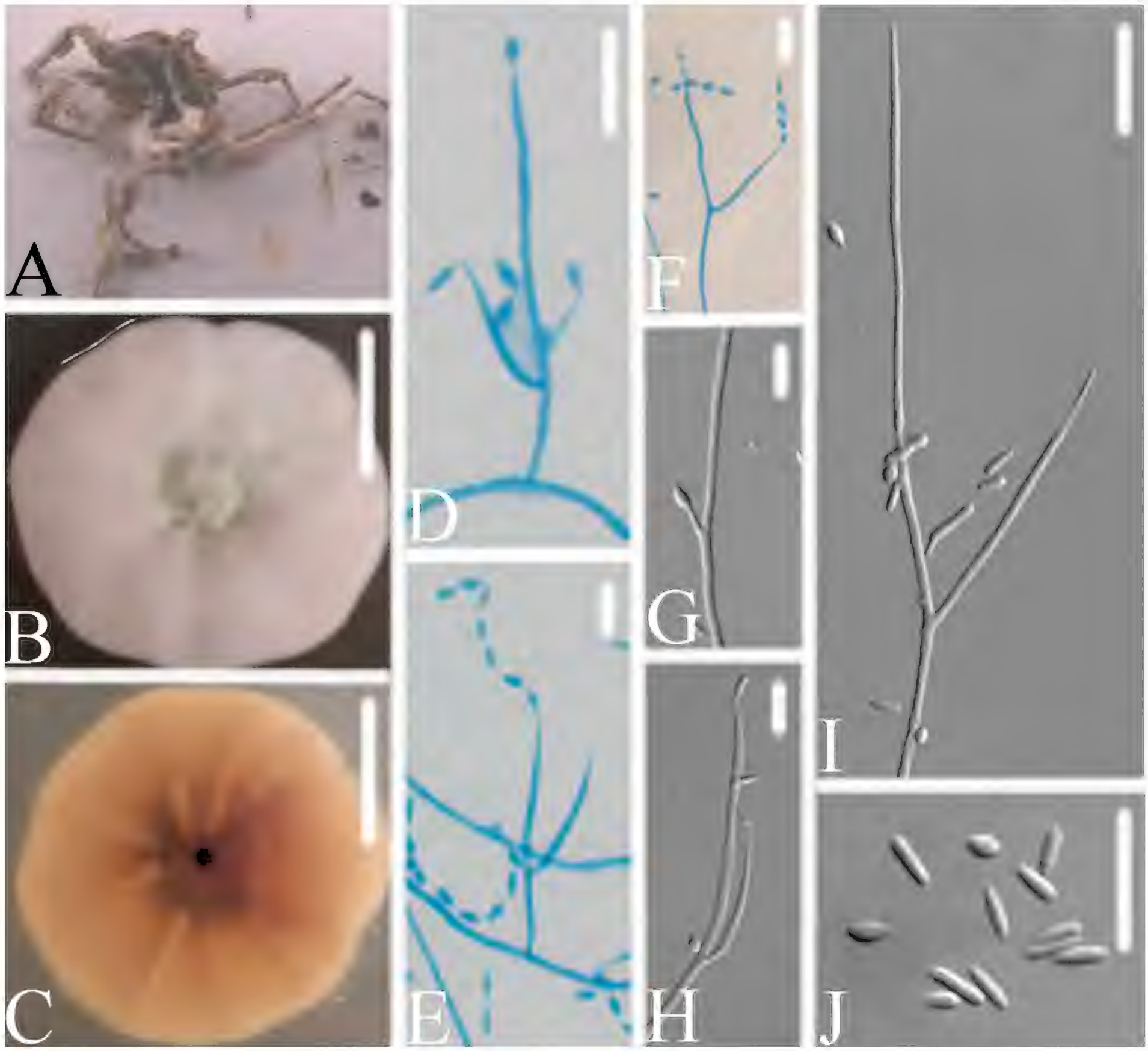

Figure 5. *Chlorocillium guizhouense* **A** infected spider **B, C** PDA culture plate showing top (**B**) and reverse (**C**) sides of the colony **D–F** phialides and conidia were stained with cotton blue **G–J** phialides and conidia. Scale bars: 10 mm (**B, C**); 10 µm (**D–J**).

Table 2. Morphological comparison of the new species with other *Chlorocillium* species.

Species	Phialides (µm)	Conidia (µm)	Hosts	Octahedral crystals	References
C. araneogenum	8.3–23.3 × 1.3–2.2	fusiform, 3.4–5.8 × 1.4–1.8	spider	Absent	Chen et al. 2022a
C. griseum	18–40(–55) × 2–2.5	fusiform, 4.5–6 × 1.0–1.5	spider	Present	Zare and Gams 2016
C. lepidopterorum	21.2–33.7 × 1.1–1.4	fusiform, 3.1–4.3 × 1.3–1.5	pupa	Absent	Chen et al. 2022a
C. sinense	11.7–20.1 × 1.1–1.3	fusiform to ellipsoidal, 1.9–2.9 × 0.8–1.2	spider	Absent	Chen et al. 2024
C. guizhouense	**11.6–25.3 × 1.0–1.2**	**fusiform, 2.6–3.8 × 1.1–1.6**	**spider**	**Absent**	**This study**
C. vallense	**9.2–17.1 × 0.8–1.2**	**fusiform, 2.0–3.2 × 0.9–1.4**	**spider**	**Absent**	**This study**

Chlorocillium guizhouense was distinguished from *C. vallense* by its larger phialides (11.6–25.3 × 1.0–1.2 µm vs. 9.2–17.1 × 0.8–1.2 µm). Thus, the morphological characteristics and molecular phylogenetic results support *C. guizhouense* as a new species.

***Chlorocillium vallense* W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.**

MycoBank No: 856176

Fig. 6

Etymology. Referring to its location, Mayao River Valley, where the fungus was first discovered.

Type. China • Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Duyun City, Mayao River Valley (26°21'24.71"N, 107°22'48.22"E). On a dead spider (Araneae), 4 September 2021, Wanhao Chen, GZAC DY0902 (holotype), ex-type, DY09021.

Description. Colonies on PDA reaching 72–74 mm diam. in 14 days at 25 °C, white, consisting of a basal felt and cottony, floccose overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 0.9–1.3 µm diam. Conidial structures consisting of erect branching conidiophores usually arising from the aerial hyphae, solitary or lecanicillium-like in whorls of two to four. Phialides 9.2–17.1 × 0.8–1.2 µm, with a cylindrical basal portion, tapering into a distinct neck. Conidia hyaline, fusiform, 1-celled, 2.0–3.2 × 0.9–1.4 µm (average values 2.4 × 1.2 µm). Octahedral crystals not observed.

Host. Spider (Araneae).

Additional strain examined. China • Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Duyun City, Mayao River Valley (26°21'24.71"N, 107°22'48.22"E). On a dead spider (Araneae), 4 September 2021, Wanhao Chen, DY09022 (living culture).

Remarks. *Chlorocillium vallense* was identified as in *Chlorocillium s. str.*, based on the BLASTn result in NCBI and the phylogenetic analysis of the

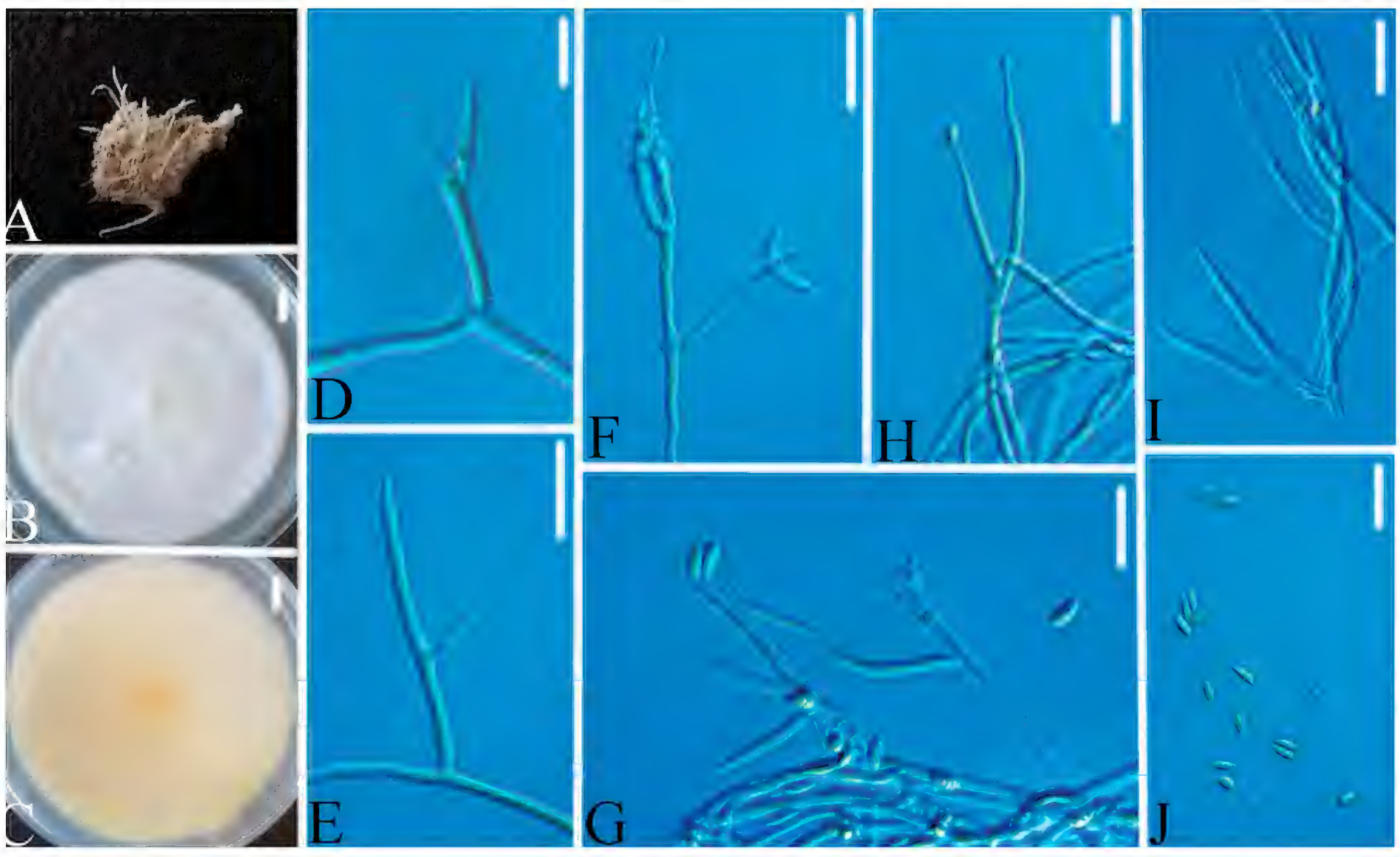

Figure 6. *Chlorocillium vallense* **A** infected spider **B, C** PDA culture plate showing top (**B**) and reverse (**C**) sides of the colony **D–J** phialides and conidia. Scale bars: 10 mm (**B, C**); 10 µm (**D–J**).

combined dataset 1 (Fig. 1) and clustered into an independent clade with a close relationship with *C. sinense* and *C. araneogenum*. Compared with the typical characteristics of the known species (Table 2), *C. vallense* was distinguished from *C. sinense* by its shorter phialide (9.2–17.1 × 0.8–1.2 µm vs. 11.7–20.1 × 1.1–1.3 µm), larger fusiform conidia (2.0–3.2 × 0.9–1.4 µm vs. 1.9–2.9 × 0.8–1.2 µm) and fast-growing in PDA. *Chlorocillium vallense* was distinguished from *C. araneogenum* by its shorter phialide (9.2–17.1 × 0.8–1.2 µm vs. 8.3–23.3 × 1.3–2.2 µm) and smaller conidia (2.0–3.2 × 0.9–1.4 µm vs. 3.4–5.8 × 1.4–1.8 µm). Thus, based on both morphological characteristics and molecular phylogenetic results, we confirm *C. vallense* as a new species.

Gamszarella Crous, Persoonia 51: 391, 2023

Gamszarella sinensis W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.

MycoBank No: 856388
Fig. 7

Etymology. Referring to the country, China, where the fungus was first discovered.

Type. China • Guizhou Province, Guiyang City, Wudang District, Bala Valley (26°45'7.0344"N, 106°58'57.09"E). On a dead spider (Araneae), 5 April 2024, Wanhao Chen, GZAC WD0408 (holotype), ex-type, WD04081.

Description. Colonies on PDA reaching 37–38 mm diam in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose overgrowth, reverse yellowish, with radial patterns. Prostrate hyphae smooth, septate, hyaline, 1.5–1.9 µm diam. Conidial structures consisting of erect conidiophores usually arising from the aerial hyphae, solitary or lecanicillium-like with conidiogenous cells in whorls of two to three. Conidiogenous cells 7.0–12.0 × 1.0–1.5 µm, with a cylindrical basal portion, bearing numerous denticles, tapering into a distinct neck. Conidia hyaline, ellipsoidal to fusiform, 1-celled, 2.4–3.9 × 1.5–2.8 µm. Octahedral crystals not observed.

Host. Spider (Araneae).

Additional material examined. China • Guizhou Province, Guiyang City, Wudang District, Bala Valley (26°45'7.0344"N, 106°58'57.09"E). On a dead spider (Araneae), 5 April 2024, Wanhao Chen, WD04082 (living culture).

Remarks. The new strains were identified as a member in *Gamszarella*, based on the BLASTn result in NCBI. The phylogenetic analyses of the combined dataset 2 (Fig. 2) showed that the new strains clustered as an independent clade with a close relationship to *Gamszarella antillana* (R.F. Castañeda & G.R.W. Arnold) Crous and *G. buffelskloofina* Crous. Compared with the typical characteristics of the known species (Table 3), the new taxon, *Gamszarella sinensis* was distinguished from *G. antillana* by its shorter conidiogenous cells bearing numerous denticles (Numerous denticles, 7.0–12.0 × 1.0–1.5 µm vs. absent of denticles, 18–31 × 1 µm), ellipsoidal to fusiform conidia and absent of octahedral crystals. *Gamszarella sinensis* was distinguished from *G. buffelskloofina* by its shorter conidiogenous cells (7.0–12.0 × 1.0–1.5 µm vs. 7–22 × 1.5–2 µm), smaller ellipsoidal to fusiform conidia [2.4–3.9 × 1.5–2.8 µm vs. (3–)4–6(–10) × 2 µm] and spider host. *Gamszarella sinensis* was distinguished from *G. vallensis* by its longer conidiogenous cells (7.0–12.0 × 1.0–1.5 µm vs. 3.8–5.4 × 1.3–1.9 µm). Thus, the morphological characteristics and molecular phylogenetic results support *G. sinensis* as a new species.

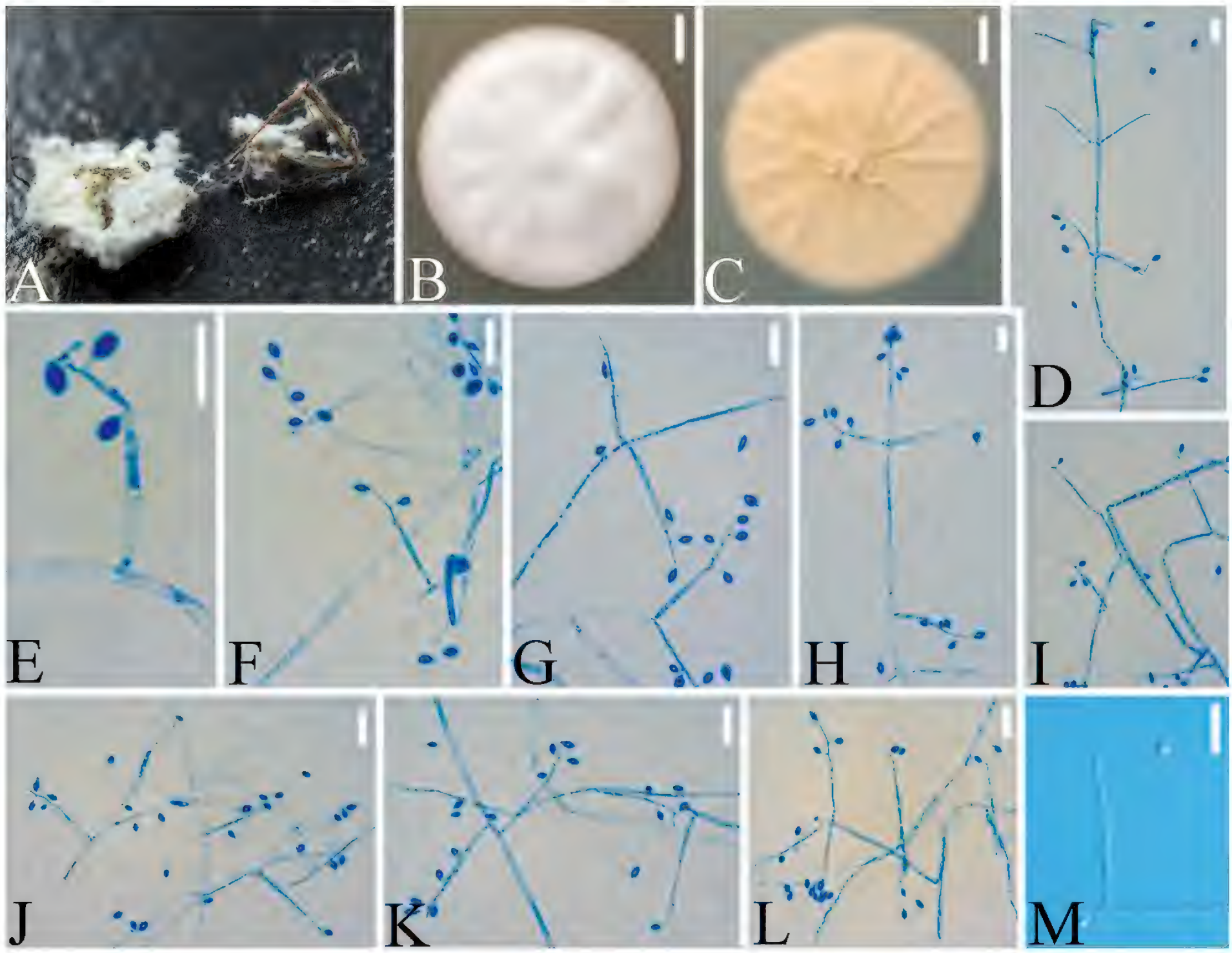

Figure 7. *Gamszarella sinensis* **A** infected spider (Araneae) **B, C** PDA culture plate showing top (**B**) and reverse (**C**) sides of the colony **D–L** conidiogenous cells and conidia were stained with cotton blue **M** conidiogenous cells and conidia. Scale bars: 10 mm (**B, C**); 10 µm (**D–M**).

Table 3. Morphological comparison of the new species with other *Gamszarella* species.

Species	Conidiogenous cells (µm)	Conidia (µm)	Octahedral crystals	Hosts/ substrate	References
G. antillana	No denticles, 18–31 × 1	Two types: primary conidia fusiform, 11–18 × 0.8–1.5; secondary conidia ellipsoidal, 3–4 × 0.8–1.2	Present	Agaric	Zare and Gams 2001
G. buffelskloofina	Terminating in a cluster of denticles, 7–22 × 1.5–2	Subcylindrical, (3–)4–6(–10) × 2	Absent	Insect	Crous et al. 2023
G. sinensis	**Numerous denticles, 7.0–12.0 × 1.0–1.5**	**Ellipsoidal to fusiform, 2.4–3.9 × 1.5–2.8**	**Absent**	**Spider**	**This study**
G. vallensis	**Numerous denticles, 3.8–5.4 × 1.3–1.9**	**Ellipsoidal to fusiform, 2.3–3.0 × 1.7–1.9**	**Absent**	**Spider**	**This study**

Gamszarella vallensis **W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.**

MycoBank No: 856389

Fig. 8

Etymology. Referring to its location, Bala Valley, where the fungus was first discovered.

Type. China • Guizhou Province, Guiyang City, Wudang District, Bala Valley (26°45'7.0344"N, 106°58'57.09"E). On a dead spider (Araneae), 5 April 2024, Wanhao Chen, GZAC WD0410 (holotype), ex-type, WD04101.

Description. Colonies on PDA reaching 25–36 mm diam in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose overgrowth, reverse yellowish, with radial patterns. Prostrate hyphae smooth, septate, hyaline, 1.5–2.0 µm diam. Conidial structures consisting of erect conidiophores usually arising from the aerial hyphae, solitary or lecanicillium-like with conidiogenous cells in whorls of two to four. Conidiogenous cells 3.8–5.4 × 1.3–1.9 µm, with a cylindrical basal portion, bearing numerous denticles, tapering into a distinct neck. Conidia hyaline, ellipsoidal to fusiform, 1-celled, 2.3–3.0 × 1.7–1.9 µm. Octahedral crystals not observed.

Host. Spider (Araneae).

Additional material examined. China • Guizhou Province, Guiyang City, Wudang District, Bala Valley (26°45'7.0344"N, 106°58'57.09"E). On a dead spider (Araneae), 5 April 2024, Wanhao Chen, WD04102 (living culture).

Remarks. *Gamszarella vallensis* was identified as in *Gamszarella*, based on the BLASTn results in NCBI. The phylogenetic analysis of the combined dataset 2 (Fig. 2) showed that the new strains clustered into an independent clade with a close relationship with *Gamszarella antillana* and *G. buffelskloofina*. Compared with the typical characteristics of the known species (Table 3), *Gamszarella vallensis* was distinguished from *G. antillana* by its shorter conidiogenous cells bearing numerous denticles (3.8–5.4 × 1.3–1.9 µm vs. 18–31 × 1 µm), ellipsoidal to fusiform conidia and absent of octahedral crystals. *Gamszarella vallensis* was

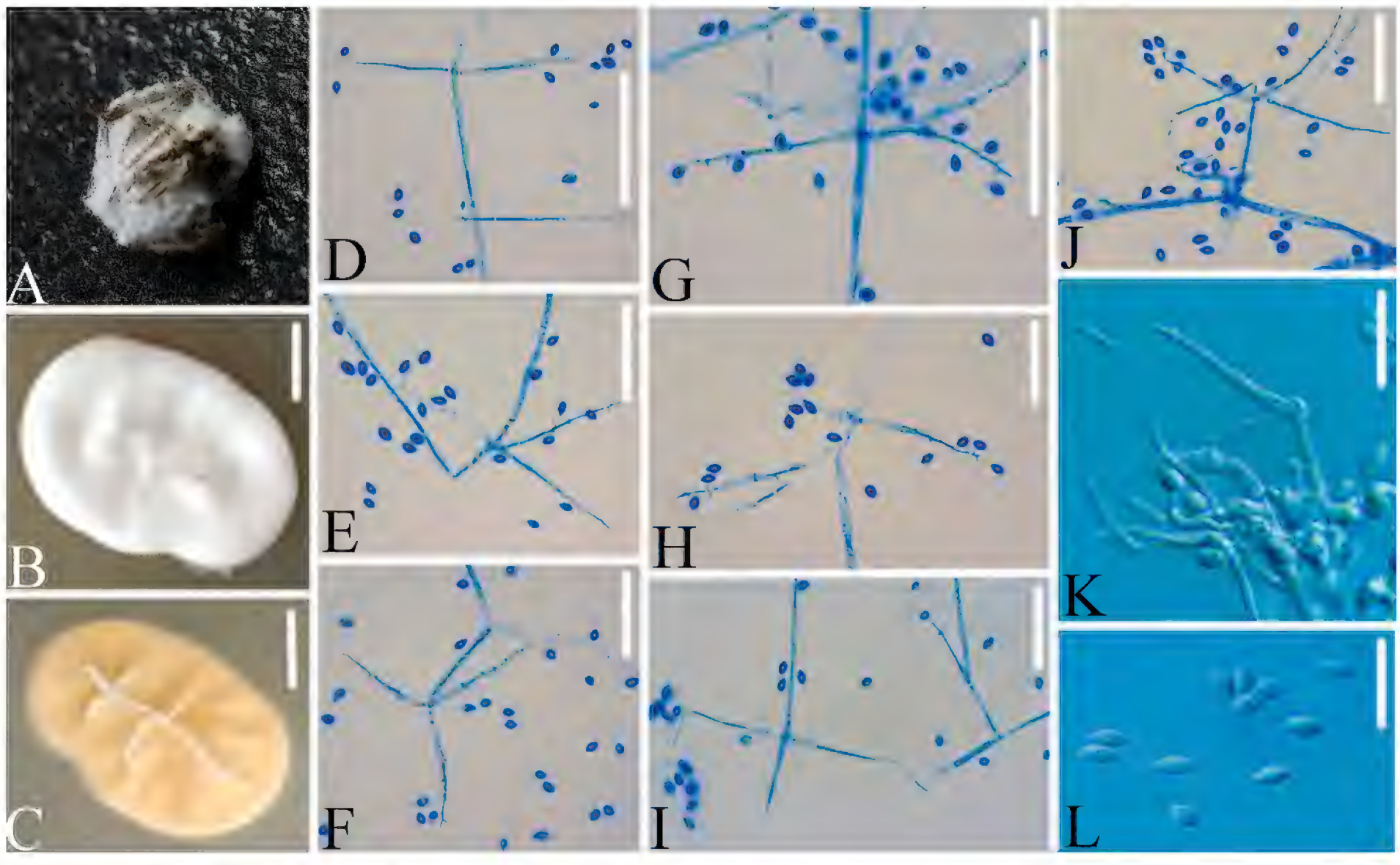

Figure 8. *Gamszarella vallensis* **A** infected spider **B, C** pda culture plate showing top (**B**) and reverse (**C**) sides of the colony **D–J** conidiogenous cells and conidia were stained with cotton blue **K, L** conidiogenous cells and conidia. Scale bars: 10 mm (**B, C**); 10 µm (**D–L**).

distinguished from *G. buffelskloofina* by its shorter conidiogenous cells (3.8–5.4 × 1.3–1.9 µm vs. 7–22 × 1.5–2 µm), smaller ellipsoidal to fusiform conidia [2.3–3.0 × 1.7–1.9 µm vs. (3–)4–6(–10) × 2 µm] and spider host. *Gamszarella vallensis* was distinguished from *G. sinensis* by its shorter conidiogenous cells (3.8–5.4 × 1.3–1.9 µm vs. 7.0–12.0 × 1.0–1.5 µm). Thus, the morphological characteristics and molecular phylogenetic results support *G. vallensis* as a new species.

Neogamszarella **W.H. Chen, Y.F. Han & J.D. Liang, gen. nov.**
MycoBank No: 856411

Etymology. Named after its morphological similarity to the genus *Gamszarella*.

Type species. *Neogamszarella antillana* (R.F. Castañeda & G.R.W. Arnold) W.H. Chen, Y.F. Han & J.D. Liang (Basionym = *Verticillium antillanum* R.F. Castañeda & G.R.W. Arnold, Feddes Repert. Spec. Nov. Regni Veg. 98 (7–8): 411, 1987).

Description. Colonies on PDA, white, with cream-colored reverse, without diffusing pigment into the agar. Conidiogenous cells developing on prostrate hyphae, single or up to 6 in verticils, subulate. Conidia solitary and of two types. Octahedral crystals present.

Host. Agaric (Hymenomycetes).

Sexual morph. Unknown.

Remarks. The genus *Gamszarella* was established with the type species *G. buffelskloofina* and two species, *G. antillana* and *G. magnispora* (Z.F. Zhang & L. Cai) Crous based on the phylogenetic analysis (Crous et al. 2023). Khonsanit et al. (2024) introduced a new genus *Corpulentispora* Khons., Thanakitp. & Luangsa-ard to accommodate *Gamszarella magnispora* based on the phylogenetic analysis and morphological characteristics. The morphological characteristics of *Gamszarella buffelskloofina* are conidiogenous cells subcylindrical with apical taper, terminating in a cluster of denticles and only one type of subcylindrical conidia (Crous et al. 2023). While the morphological characteristics of *Gamszarella antillana* are conidiogenous cells absent of denticles and produce two types of fusoid conidia, macroconidia and microconidia. *G. antillana* do not fit with the genus *Gamszarella*. Thus, a new genus is proposed to accommodate species *Gamszarella antillana*.

Neogamszarella antillana **(R.F. Castañeda & G.R.W. Arnold) W.H. Chen, Y.F. Han & J.D. Liang, comb. nov.**
MycoBank No: 856412

= *Verticillium antillanum* R.F. Castañeda & G.R.W. Arnold, Feddes Repert. Spec. Nov. Regni Veg. 98 (7–8): 411, 1987. Basionym.
= *Lecanicillium antillanum* (R.F. Castañeda & G.R.W. Arnold) Zare & W. Gams, Nova Hedwigia 73(1–2): 34, 2001.
= *Gamszarella antillana* (R.F. Castañeda & G.R.W. Arnold) Crous, Persoonia 51: 391, 2023.

Remarks. *Verticillium antillanum* was transferred to the genus *Lecanicillium* by Zare and Gams (2001). Crous et al. (2023) introduced a new genus *Gamszarella*

and combined *Lecanicillium antillanum* with *Gamszarella* based on the phylogenetic analysis. The morphological characteristics of *Gamszarella antillana* were similar to *Corniculantispora aranearum* (Petch) Khons. et al., *C. dimorpha* (J.D. Chen) Khons. et al., *C. psalliotae* (Treschew) Khons. et al., *Flavocillium acerosum* (Zare & W. Gams) H. Yu et al., and both species produce two types of conidia (Wang et al. 2020; Khonsanit et al. 2024). However, *Gamszarella antillana* was distinguished from *Corniculantispora aranearum*, *C. dimorpha*, *C. psalliotae* and *Flavocillium acerosum* by the phylogenetic analysis (Fig. 2). Besides, the morphological characteristics of *Gamszarella antillana* do not fit with the typical characteristics of the genus *Gamszarella*. Thus, *Gamszarella antillana* was combined into the new genus *Neogamszarella* as *Neogamszarella antillana*.

Discussion

Karst regions in southwestern China are one of the world's 36 biodiversity hotspots, home to a wide range of endemic species (Delgado-Baquerizo et al. 2020). Wijayawardene et al. (2021) discussed the necessity of systematic studies to reveal novel taxa in Yunnan–Guizhou Plateau. Many new entomopathogenic fungi were found in the Kasit regions of Yunnan and Guizhou Provinces (Peng et al. 2023, 2024; Tang et al. 2023a, b; Xiao et al. 2023, 2024; Zhang et al. 2023; Chen et al. 2024; Dai et al. 2024; Fan et al. 2024; Wang et al. 2024b). Besides, there is high spider diversity in the Karst regions, especially in caves (Zhang and Li 2014; Liu et al. 2023).

The present study introduces four new species of *Chlorocillium* and *Gamszarella* from spiders. *Chlorocillium* species are often found on spiders, aphids and scale insects (Zare and Gams 2016). The taxonomic delimitation of *Chlorocillium* was originally based on morphological characteristics and phylogenetic analysis of ITS or LSU sequences (Zare and Gams 2016). Tan and Shivas (2023, 2024) reported two new species based on phylogenetic analysis of ITS, LSU, *RPB2*, and *tef*-1α sequences. In this study, we introduce two new species of *Chlorocillium* viz., *C. guizhouense* and *C. vallense*, based on both morpho-molecular data (ITS, LSU and *tef*-1α) (Figs 1, 5, 6). Moreover, PHI test was carried out to visualize the differences among *Chlorocillium* species, and supports the results of morphological characteristics and phylogenetic analysis (Fig. 3). Therefore, combined analysis of morphological characteristics, phylogenetic analysis and other methods may benefit the taxonomy of *Chlorocillium*.

Crous et al. (2023) introduced *Gamszarella* with the type species *G. buffelskloofina* and transferred *Lecanicillium antillanum* (Castañeda & G. Arold) Zare & W. Gams and *L. magnisporum* Z.F. Zhang & L. Cai into *Gamszarella* based on the phylogenetic analysis and morphological characteristics. Khonsanit et al. (2024) proposed a new genus *Corpulentispora* Khons. et al. to accommodate *Gamszarella magnispora*. In the present study, two new species of *Gamszarella* viz., *G. sinensis* and *G. vallensis*, are introduced based on both morpho-molecular data (ITS, LSU, *RPB2* and *tef*-1α) (Figs 2, 7, 8). However, the morphological characteristics of *Gamszarella antillana* were significantly different from *G. buffelskloofina*, *G. sinensis* and *G. vallensis* by its two types of conidia, no denticles, presence of octahedral crystals and Agaric substrate. Thus, we proposed a new genus, *Neogamszarella*, to accommodate this species.

Our study confirms the high fungal diversity associated with arthropods in South-Western China. Nevertheless, fungi associated with spiders are poorly known and need thorough, systematic exploration.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Data curation: WHC. Formal analysis: JDL. Funding acquisition: WYT, YFH, JHZ, WHC. Methodology: YFH. Resources: WHC. Writing - original draft: HLS, WHC, JDL, DL. Writing - review and editing: WYT, YFH, JHZ.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

The genera of araneogenous fungi in the order Hypocreales

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